Cold-Shock expression vector pCold™ ProS2 DNA

Code No. 3371

Size: 25 μ g (0.5 OD)

Shipping at − 20 °C Stored at − 20°C

Lot No.

Concentration:

 $0.5 \mu g/\mu l$

Volume:

 μ I

Regarding protocol, please refer to the product manual supplied with pCold™ ProS2 DNA.

Description:

Description:

Cold-shock expression vector, pCold™ ProS2 DNA, is a fusion expression vector utilizing a promoter derived from cspA gene, which is one of the cold-shock protein, and fused tandem N-terminal domain of Protein S, which is a kind of Myxococcus xanthus genes, as a solubility promoting tag, ProS2. At the downstream of the cspA promoter, Iac operator is inserted so that the expression is strictly controlled. In addition, 5' untranslated region (5' UTR), translation enhancing element (TEE), His-Tag equipme. ProS2 sequence and multiplication (its (MCS) are located at sequence, ProS2 sequence, and multicloning site (MCS) are located at

the downstream of the *cspA* promoter. Between ProS2 sequence and MCS, HRV 3C Protease, Thrombin and Factor Xa cleavage sites are inserted so that the fusion tag can be removed

from the fusion expression protein.

As this product utilizes the promoter drived from E. coli, most E. coli strains can be utilized as an expression host.

Form: 10 mM Tris-HCl, pH8.0 1 mM EDTA

Length: 5,025 bp

Purity:

- Contains over 70% double-stranded covalently closed circular DNA (RF I). Confirmed to maintain cloning sites by dideoxy sequencing method. Shown to be cleaved at a single site by restriction enzymes Nde I, Sac I, Kpn I, Xho I, BamH I, EcoR I, Hind III, Sal I, Pst I and Xba I.

Applications:

This is a vector for protein expression utilizing the promoter of cold shock gene (cspA).

Vector map: (See the reverse side.)

Note:

The use of this product is limited for research purposes. It must not be used for clinical purpose or for *in vitro* diagnosis.

Users must contact TAKARA when they plan to use this product for pur-

poses other than research use.

- 1. PRODUCTS are for research or laboratory use only. PURCHASER understands and agrees that PRODUCTS shall not be administered to humans or animals, and shall not be used for pharmaceutical, in vitro diagnostic, or any commercial purposes other than internal research.
- 2. PURCHASER shall not modify pCold Vector DNA sequences located between and including the CspA 3'UTR and CspA Promoter. The adjacent multicloning site (MCS) is except from this restriction.
- 3. PURCHASER shall not utilize any partial sequences from PRODUCTS for the purpose of new plasmid construction using the Cold Shock
- Expression System.

 4. PURCHASER shall not transfer or sell copies of PRODUCTS, components of PRODUCTS, derivatives of PRODUCTS, and/or products obtained through the use of PRODUCTS (collectively "DERIVATIVES") to any third parties. Notwithstanding foregoing, PURCHASER may transfer DERIVATIVES solely to a third party which has already been granted a license to use PRODUCTS for research purpose through purchase of PRODUCTS from TAKARA BIO, its subsidiaries or its local distributors, provided that PURCHASER shall enter into a prior separate agreement with TAKARA BIO are the transfer of DERIVATIVES to said third party. with TAKARA BIO for the transfer of DERIVATIVES to said third party.

NOTICE TO PURCHASER: LIMITED LICENSE

[L13] pCold vectors

This product is covered by the claims of U.S. Patent No.5,981,280, 6,686,174, 6,333,191 and their foreign counterpart patent claims, assigned to the UMDNJ. This product is covered by the claims of U.S. Patent No. 6,479,260, 6,897,042 and their foreign counterpart patent claims.

[L16] His-Tag Sequence
This product is covered by the claims of U.S. Patent No. 5,284,933, 5,310,663 and their foreign counterpart patent claims. Protein Purification Technology of His-Tag used in some of pCold vectors is licensed from Hoffmann-La Roche, Inc., Nutley, NJ and/or Hoffmann-La Roche Ltd., Basel, Switzerland and is provided only for the use in resaerch. Information about licenses for commercial use is available from QIAGEN GmbH, Qiagen Strasse 1, D-40724 Hilden, Germany.

[L43] Protein S

This product is the subject of the pending U.S. patent application and its foreign counterparts.

[M9] pCold vectors

This product is covered by the claims of U.S. Patent No. 6,479,260 and its foreign counterpart patent claims.

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Produced by TAKARA BIOTECHNOLOGY (DALIAN) CO. LTD. Cloning site of pCold ProS2 DNA

Cionning site of peola Prosz DNA.			
	5' TAACGCTTCAA	AATCTGTAAAGCACGCCAT	TATCGCCGAAAG
	TEE	His-Tag	
GCACACTTAATTATTAA <u>GAGG</u> TAATACACCATGAATCACAAAGTGCATCATCATCATCAC			
SD Me	et Asn His Lys Val H	is His His His His	
ATGProS2 Tag (552 bp)GTCTCCAGCATCCGCGTCATCTCCGTGCCGGTGCAGCCGAGG MetProS2 Tag (184 aa)Val Ser Ser Ile Arg Val Ile Ser Val Pro Val Gin Pro Arg			
pCold-PrS2-F2 primer		pCold-PrS2-F1	primer
HRV 3C Protease		Thrombin L	Factor Xa
TCCGCGGGTCTGGAAGTTCTGTTCCAGGGGCCCTCCGCGGGTCTGGTGCCACGCGGTAGTGGTGGTATCGAAGGTAGG Ser Ala Gly Leu Glu Val Leu Phe Gln Gly Pro Ser Ala Gly Leu Val Pro Arg Gly Ser Gly Gly Ile Glu Gly Arg			
Nde I Sac I Kpn I Xho I BamH I EcoR I Hind III Sal I Pst I Xba I CATATG GAGCTC GGTACC CTCGAG GGATCC GAATTC AAGCTT GTCGAC CTGCAG TCTAGA TAGGTAATCTCTGCT His Met Glu Leu Gly Thr Leu Glu Gly Ser Glu Phe Lys Leu Val Asp Leu Gln Ser Arg End			
pCold-R primer		•	
TAAAAGCACAGAATCTAAGA <u>TCCCTGCCATTTGGCGGGGA</u> TTTTTTTTTTTTTTTCAGGAAATAAATAATCGAT 3'			
transcription terminator			

v200908Da

コールドショック発現ベクター pCold® ProS2 DNA

Code No. 3371

Size : 25 μ g (0.5 OD)

Shipping at -20° C Stored at -20° C

Lot No.(英文面をご覧ください。)濃度:(英文面をご覧ください。)容量:(英文面をご覧ください。)

本酵素の使用方法については、製品添付の取扱説明書をご確認ください。

●製品説明

pCold® ProS2 DNA は、*Myxococcus xanthus* に由来する Protein SのN 末端ドメインを2つタンデムに繋いだ ProS2 タグを、可溶化タグとして使用する融合型のコールドショック発現ベクターである。大腸菌コールドショック遺伝子の一つである *cspA* 遺伝子のプロモーターを利用しており、*cspA* プロモーターの下流に5′非翻訳領域(5′UTR)、translation enhancing element (TEE)、His タグ配列、ProS2 タグ配列、multicloning site などが配置されている。また、プロモーターの下流には発現を厳密に制御するための *lac* operator が挿入されている。

ProS2 タグと MCS の間には HRV 3C Protease、Thrombin、Factor Xa の認識配列があり、発現後の融合タンパク質からタグを除去することができる。pCold® ProS2 DNA は大腸菌のプロモーターを用いているため、ほとんどの大腸菌株を発現宿主として利用することができる。

●形状 10 mM Tris-HCl, pH8.0 1 mM EDTA

●鎖長 5,025 bp

●純度

- 1. 二重鎖 covalently closed circular form I(RFI)を70%以上含んでいる。
- 2. dideoxy 法によるシーケンスの結果、クローニングサイトが保持されていることを確認している。
- 3. 制限酵素 Nde I、Sac I、Kpn I、Xho I、BamH I、EcoR I、Hind III、Sal I、 Pst I、Xba I にて 1 ヵ所切断できることを確認している。

●用途

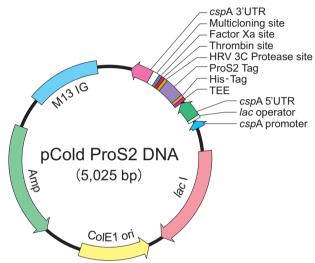
コールドショック遺伝子 (cspA) のプロモーターを利用したタンパク発現。

● pCold® ProS2 DNA のクローニングサイト図

(英文面をご覧ください)

● pCold® ProS2 DNA のベクターマップ

(Vector map of pCold® ProS2 DNA)



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 - 2) New Jersey 医科歯科大学 Protein S テクノロジーのライセンス: Code 3371
 - 3)QIAGEN 社 His・Tag テクノロジーのライセンス:Code 3360, 3361, 3362, 3365, 3371
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